

Supporting Text

Calculation of Relative Conductances of Hemichannels and Cell–Cell Channels

Given a simple series arrangement of hemichannels to form cell–cell channels (Fig. 6), the measured conductance of the open cell–cell channel, G_{o-cell} , should be about half the measure conductance of the open hemichannel, G_{o-hemi} , with a correction for access resistance, g_a . Let g_o and g_s be the open and substate conductances of a hemichannel in series with the access conductance. If g_a is large compared to g_o ,

$$\begin{aligned}G_{o-hemi} &= g_o g_a / (g_a + g_o) \approx g_o \\G_{o-cell} &= g_o g_a / 2(g_a + g_o/2) \approx g_o/2.\end{aligned}$$

The resistance of a cylindrical channel plus the sum of access resistances on the two sides is approximated by $(L + \pi a/2)\rho/(\pi a^2)$, where L is channel length, a is channel radius, and ρ is resistivity of the cytoplasm (1). Thus, access resistance increases single-channel resistance by a factor of 1.6 times the ratio of channel radius to channel length. For a gap junction channel, the channel radius is 0.5–0.75 nm, the length is ~10 nm, and access conductance is >10 times the cell–cell channel conductance and >5 times the hemichannel conductance. The ratio of G_{o-hemi} to G_{o-cell} including access resistance is given by $2(g_a + g_o/2)/(g_a + g_o)$, which is ~1.9 for an access conductance that is 10 times the cell–cell channel conductance. The observed values of

$$\begin{aligned}G_{o-hemi} &= 220 \text{ pS} \\G_{o-chan} &= 110 \text{ pS} \approx 1/2 G_{o-hemi}\end{aligned}$$

are in reasonable agreement with these approximations.

We also predict that the conductance of the substate of the cell–cell channel, G_{s-chan} , should be the series sum of the open and substate conductances of the hemichannel and the access conductance, because only one of the two series hemichannels will be closed to the substate by V_j gating. The observed conductance of the substate of the hemichannel in series with the access conductance, that is G_{s-hemi} , was ~75 pS, which would be only minimally smaller than the “true” hemichannel substate conductance, g_s . We can calculate G_{s-chan} using this value of G_{s-hemi} to approximate g_s :

$$G_{s-chan} = G_{o-hemi} g_s / (G_{o-hemi} + g_s) = 75 \times 220 / (75 + 220) \approx 56 \text{ pS}.$$

The reported value of G_{s-chan} is ~30 pS, which is much lower than this calculated value. The calculation can be made in the opposite direction by using observed values of G_{s-chan} and G_{o-hemi} . Solving the preceding equation for g_s ,

$$g_s = G_{s-chan} G_{o-hemi} / (G_{o-hemi} - G_{s-chan}) = 220 \times 30 / (220 - 30) \approx 35 \text{ pS}.$$

This value is markedly smaller than the observed value of 75 pS.

Calculation of Time Constant of Equilibration of Dye Influx as a Function of Single Hemichannel Permeability and Number of Channels

We wish to estimate the time constant of dye influx through hemichannels into cells expressing connexin 43-enhanced GFP (Cx43-EGFP). For gap junctions between teleost blastomeres, total permeability to tetraethyl ammonium ions is proportional to conductance with a proportionality constant of $10^{-3} \text{ cm}^3 \cdot \text{sec}^{-1}$ per S (2). Taking this value as an approximation for ethidium bromide permeation, the permeability, $P_{j\text{-hc}}$, for n hemichannels with a conductance of 220 pS is

$$P_{j\text{-hc}} = 220n \times 10^{-15}.$$

The time constant of equilibration for a cell with volume, V , bathed in a much larger volume of dye solution is

$$\tau = V/(nP_{i\text{-hc}}).$$

For a cell approximated by a 10- μm cube,

$$\begin{aligned} \tau &= 10^{-9} / (220n \times 10^{-15}) \\ &= 4.5 \times 10^3 / n \text{ sec} \\ &= 1.25 / n \text{ h.} \end{aligned}$$

Using instead the treatment in ref. 3 for diffusion in an aqueous channel, the total permeability is related to number of channels, n , diffusion coefficient, D , pore radius, r , and pore length, L , as

$$P_{i\text{-hc}} = nD\pi r^2/L.$$

The value of D for ethidium bromide is $\sim 4 \times 10^{-6} \text{ cm}^2/\text{s}$ (4), r is $\sim 7.5 \times 10^{-8} \text{ cm}$, and L_{pore} is $\sim 10^{-6} \text{ cm}$, from which

$$\begin{aligned} P_{i\text{-ch}} &= 7.1n \times 10^{-14} \\ \tau &= 1.4 \times 10^4 / n \text{ sec} \\ &= 3.8 / n \text{ h.} \end{aligned}$$

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